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LIQUID HANDLING SYSTEM

TECHNICAL FIELD

The present invention relates to microfluidic devices comprising microchannels, and to methods for replacing solvent amounts that evaporate from open microareas carrying microvolumes of the solvent. The microvolume of solvent may be in the form of a droplet (microdrop).

BACKGROUND ART

Microvolume handling systems have attracted a considerable interest in biochemical analysis, combinatorial chemistry and high throughput screening (HTS) applications. The miniaturised format is compatible in size with many interesting issues of bioanalytical work, such as single cell analysis, when material is available only in extremely limited amounts. Furthermore by decreasing the volume, an enhanced efficiency in terms of a higher rate of mixing and/or chemical reaction can be expected in the sample container, since the effect of diffusion and thermal convection is more pronounced on a smaller scale.

In HTS applications, goals are currently set on screening more than 10^5 compounds in a single assay. To manage such a tremendous number of samples with reasonable space, cost and time requirements, the miniaturised microtitre plate format has been developed. Based on micromachining of different materials, e.g., by anisotropically etching single crystalline silicon wafers, well-defined picolitre to nanolitre vials are readily fabricated (Jansson et al. (1992) J. Chromatography 626, 310-314; Beyer Hietpas et al. (1995) J. Liq. Chromatography 18, 3557-3576). Biomolecules such as DNA and prot ins have been assayed in the microvial format utilising capillary electrophoresis (Jansson t al. *supra*; Beyer Hietpas et al., *supra*), bioluminescence (Crofcheck et al. (1997) Anal. Chem. 69, 4768-4772), electrochemical analysis (Clark et al. (1997) Anal. Chem. 69, 259-263; Clark et al.

(1998) Anal. Chem. 70, 1119-1125) and mass spectrometry (Jespersen et al. (1994) J. Rapid Comm. in Mass Spectrom. 8, 581-584).

However, the rate of solvent evaporation is particularly pronounced for microvolumes, for instance small droplets, since the surface-to-volume ratio increases when the drop diameter decreases. The most common way for avoiding desiccation is by covering the containers with a material non-permeable for the underlying solvent. However, covers, either liquid or solid, inherently has a potential of introducing interfering compounds or altering equilibrium that seriously can damage sensitive chemical systems. Furthermore, practical problems may arise from small droplets sticking to a solid cover.

An alternative is to diminish the solvent loss by controlling the environment in humidified chambers and by dispensing compensating solvent into the microvials *via* fine capillaries from above (Roeraade et al. (1996) Analytical Methods and Instrumentation. Special issue μTAS'96 (1996), pp. 34-38). However, this technique can be ineffective over prolonged time periods and is subject to many practical problems associated with a restricted accessibility to the vials through the environmental chamber. Furthermore, since the solvent compensating capillaries blocks the space in close proximity to the microvials, accessing or detecting the material becomes increasingly more complex the larger the assay.

There is a need for microfluidic devices including a system for handling small volumetric amounts of liquid which avoids the above discussed drawbacks and allows for free access to the contained material, thus facilitating chemical manipulation of the liquid or the gaseous headspace environment and for monitoring of reaction products.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic view of a system for containing small amounts of material in a droplet on top of the orifice of a microchannel.

Figure 2 is an enlarged view of the top of the capillary in Figure 1 illustrating a droplet.

Figure 3 illustrates the three different possible shapes of the liquid-gaseous interface.

Figure 4 illustrates a top of a microchannel with a droplet and sample components immobilised on the microchannel rim.

Figure 5 illustrates a circular array of fabricated holes containing microdrops.

Figure 6 is a sectional view of Figure 4 illustrating a solvent container.

Figure 7 is a schematic view of a rectangular array

Figure 8 is a sectional view of Figure 7

DISCLOSURE OF THE INVENTION

The present invention provides a method for replacing solvents evaporating from a microvolume of solvent placed in an open microarea of a microfluidic device. The method has the characterizing feature that replacement is continuously taking place via a microchannel that transports liquid to the microarea from a liquid reservoir (vessel). The method is particularly useful in the context of running reactions in the solvent present on the microarea in order to assay an analyte, for the synthesis of chemical compounds etc. The reactants used, including an analyte and/or various reagents, may be soluble in th microvolume or immobilized to a solid support in contact with the microvolume. The microarea may be the orifice region of the microchannel and the microvolume in the form of a microdrop, as shown in Figure 2 (1). By continuously replacing the evaporated solvent *via* a conduit (2) with solvent from a communicating vess l (3) the reactants present in the microvolume are

prevented from being desiccated. The sample is focused in the microvolum as long as the evaporation rate of the solvent is higher than the sample diffusion. It should be noted that the solvent compensating principle is generally applicable to minute volumes, thus the liquid-gaseous interface may appear in any of the different shapes illustrated in Figures 3 a-c. In the case of droplets (figure 3 a), they are formed by applying an overpressure to the solvent supplying tubing. This causes the droplet size to be determined by the diameter of the capillary orifice, the interfacial tension, the wettability of the capillary material and the magnitude of the applied overpressure (which needs to be in equilibrium with the interfacial pressure difference across the curved surface of the droplet). The microarea can be located either, as illustrated in Figure 1, on top of a single capillary (4), or (Figures 5 - 8) as an array of microareas carrying liquid in form of drops (6) or liquid in form of other physical microappearances (9) (e.g. surfaces of the type shown in figures 3 b-c) formed on top of an array of fabricated holes (7) each supplied from a common solvent container (8). In the case of droplets, the needed overpressure can be created by any means of pressure generation, e.g. from a hydrostatic head, a micropump or a pressurised container.

The open geometry in this invention, with microareas carrying analyte- and/or reagent-containing solvent in direct contact with the surrounding gaseous phase, is favourable with respect to the free accessibility. For example, wet-chemical reactions can easily be performed with sample components contained in the surface layers, using reagents dispensed from external means directly to the microvolume of liquid placed in the microarea, for instance from ink-jet dispensers or fine pipettes. Furthermore, detection of analytes or reaction products can readily me made using optical detectors, such as CCD-cameras. Moreover, the equilibrium between the solvent on the microarea and the surrounding gaseous phase could be exploited for passive sampling of air-born constituents over prolonged time periods, thus enabling subsequent environmental analysis.

The solvents contemplated ar often aqueous, i.e. consists of water, possibly mixed with one or more water-miscible liquids, such as acetone, methanol, ethanol and isopropanol. This does not exclude the us of other solv nts in the invention.

A second aspect of the invention relates to a microfluidic device comprising a microchannel providing for liquid contact between an open microarea carrying a microvolume of a solvent and a reservoir for the solvent, said reservoir and said microchannel being adapted so that solvent evaporated from said microarea is continuously replaced by solvent from the reservoir through said microchannel. When in use the microvolume of solvent typically contains an analyte and/or one or more reagents for assaying the analyte either directly or indirectly, for running synthesis of a compound etc. By the term "indirectly" is contemplated that a feature or an amount of a reaction product related to the analyte is assayed.

In order to avoid the risk of desiccation of the microareas over prolonged time periods, the supplying solvent vessel should contain a solvent volume one, two three or more orders of magnitude larger than the sum of all microvolumes communicating with the reservoir.

By "microfluidic device" is meant a device that can handle microvolumes of reagents, for example volumes less than 1 µl, preferably between 1 and 10 nl, which may be introduced into the device.

The term "microvolume" means a volume that typically is at most around 10 µl, such as ≤ 1 µl . The lower end of the range extends down to the infinitesimal volume that is present in the gaseous-liquid interface of the microvolume of the solvent. Typically the microvolume is ≥ 10^{-15} l (femtolitre). It will be understood, however, that the described principles may be applicable also to microvolumes being larger than 10 µl .

A microarea may have different forms from an essentially flat form via cup-formed areas to walls of open chambers, the important matter being that the area is able to carry the microvolume of liquid contemplated.

Microchannels typically have the ability to act as capillaries. Normally their size in the smallest dimension is less than 2000 μm, such as ≤ 500 μm. Typically this dimension is ≥ 1 μm. A microchannel may be in form of a tube that may have a circular, a rectangular etc cross sectional area. They may also be "sheet"-like covering larger areas.

The reagents included in or in contact with the microvolume of solvent varies depending on the reaction to be run. The reagents include catalysts, for instance an enzyme, compounds needed for the synthesis of nucleic acids, affinity reactants etc. The term also includes biological systems, such as enzymatic systems and whole cells. Affinity reactants typically form non-covalent complexes and may be illustrated by biotin, streptavidin, protein A, antibodies, lectins, hormone receptors, nucleic acids, peptides and polypeptides. Typical assays are immunoassays, sequenceing of nucleic acids and of peptides, hybridization assays, detection of mutations, cell assays, etc.

In one aspect of the invention one or more of the reagents used are immobilised in the microarea. This alternative configuration is illustrated in Figure 4, where reagents are immobilised on the rim (5) of a microchannel, allowing washing steps to be performed by overflowing the microchannel. Immmobilization may be via covalent bonds, affinity bonds, physical adsorption etc. Typical affinity bonds are those forned by having strepavidin or a high affinity antibody bound to a solid support in the microarea and then binding a desired reagent conjugated with biotin or with the hapten against which the antibody is specific to the solid support bound strepavidin/high affinity antibody.

The microfluidic device according to the invention can suitably be fabricated in the form of a circular (Figure 5 and 6) or rectangular array format (Figure 7 and 8).

A circular format means that there are one or more microareas (chambers) that are placed radially and in different directions from a center. The distance from the center to individual microareas (chambers) may be equal or different. The reservoir is preferably in the center. The microchannels may be radially directed from th

center and communicate with one or more microareas. The microchannels may also be in the form of a common flat-like microchannel or reservoir beneath the microareas (chambers) and communicating upwardly via traditional microchannels.

In rectangular formats there are microareas (chambers) that form a rectangular pattern. The microchannel arrangement may be in analogy with the circular format.

Microfluidic devices in the form of rotatable discs are known in the art. WO 97/21090 discloses a microanalytical / microsynthetic system for biological and chemical analysis, comprising a rotatable microplatform, e.g. a disc, having inlet ports, microchannels, detection chambers (microareas) and outlet ports through which fluid may flow. Preferably a circular array comprises a disc and a plurality of microchannels (see Figure 5 and 6), each microchannel being radially dispersed about the centre of the said rotatable disc. The rotatable disc is adapted for rotation about its axis. Such adaptation may take the form of a hole at the axis of one or both substrates which is capable of engaging a drive shaft. Other methods of rotating the disc include clamping the disc and contacting the perimeter with a moving surface, for example moving wheels, or placing the disc on a turntable and spinning the turntable. Preferably the disc comprises a solvent inlet port located towards the centre of the disc, *via* radially dispersed microchannels, each microchannel having an sample reservoir located at the microchannel orifice.

The configuration of the microchannels in the rectangular or circular format is may be chosen to allow for application of a chemical compound, or a suspension of cells, to the sample reservoir filled with fluid medium.

The microfluidic device may also comprise a separate microchannel system for transporting one or more of the reactants needed to the microareas.

Suitably the circular or rectangular array format is a one- or two-piece construction assembled together to provide a closed structure with openings at defin d positions to allow loading of the d vice with liquids and removal of waste liquids. In the simpl st form, the disc is produced as two complementary parts, on or each

carrying channel structures which, when affixed together, form a series of interconnected structures within the body of a solid disc or wafer. The microchannels may be formed by micro-machining methods in which the channels and chambers are micro-machined into the surface of a disc or wafer, and a cover, for example a plastic film, is adhered to the surface so as to enclose the channels and chambers.

Suitable glass or polymeric materials can be additionally selectively modified by chemical or physical means to alter the surface properties to confer a desired property, e.g. compatibility with cell growth, cell attachment and the attachment of biomolecules by covalent or non-covalent means.

Based on knowledge at the priority date, the variant given in figures 1 and 2 corresponds to the best mode in October 1998.

CLAIMS

1. A microfluidic device comprising a microchannel providing for liquid contact between an open microarea carrying a microvolume of a solvent and a reservoir for the solvent, said reservoir and said microchannel being adapted so that solvent evaporated from said microarea is continuously replaced by solvent from the reservoir through said microchannel.

2. The microfluidic device according to claim 1 wherein
   a) said reservoir is positioned so as to create an overpressure in the solvent which is in equilibrium with the interfacial pressure difference across the curved surface of the droplet, or
   b) said reservoir is connected to pump means that either facilitate replacement of solvent by pumping solvent or pressurising the reservoir.

3. The microfluidic device according to anyone of claims 1-2 comprising a plurality of microchannels and open chambers forming an array in the circular or rectangular format.

4. The microfluidic device according to anyone of claims 1-3, wherein the microvolume contains one or more reactants that are soluble in the solvent or bound to a solid support in contact with the microvolume.

5. The microfluidic device according to claim 4 wherein at least one of said one or more reactants is an affinity reactant, for instance selected from nucleic acids, peptides, proteins.

6. A method for replacing solvents evaporating from a microvolume of solvent plac d in an open microarea of a microfluidic device, characterized in that that replacement is continuously taking place via a microchannel that transports liquid to the microarea from a liquid reservoir (vessel).

7. The method of claim 6, characterized in that the microarea, microchannel and reservoir are parts of the microfluid device defined in claims 1-5.

ABSTRACT

A microfluidic device comprising a microchannel providing for liquid contact between an open microarea carrying a microvolume of a solvent and a reservoir for the solvent, said reservoir and said microchannel being adapted so that solvent evaporated from said microarea is continuously replaced by solvent from the reservoir through said microchannel.

A method for replacing solvents evaporating from a microvolume of solvent placed in an open microarea of a microfluidic device, characterized in that that replacement is continuously taking place via a microchannel that transports liquid to the microarea from a liquid reservoir (vessel).

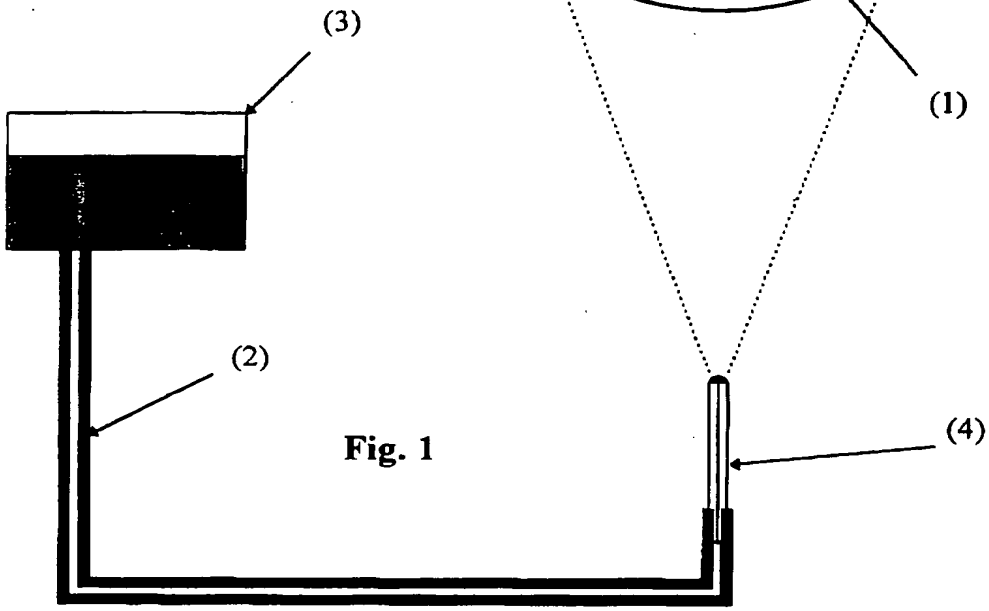
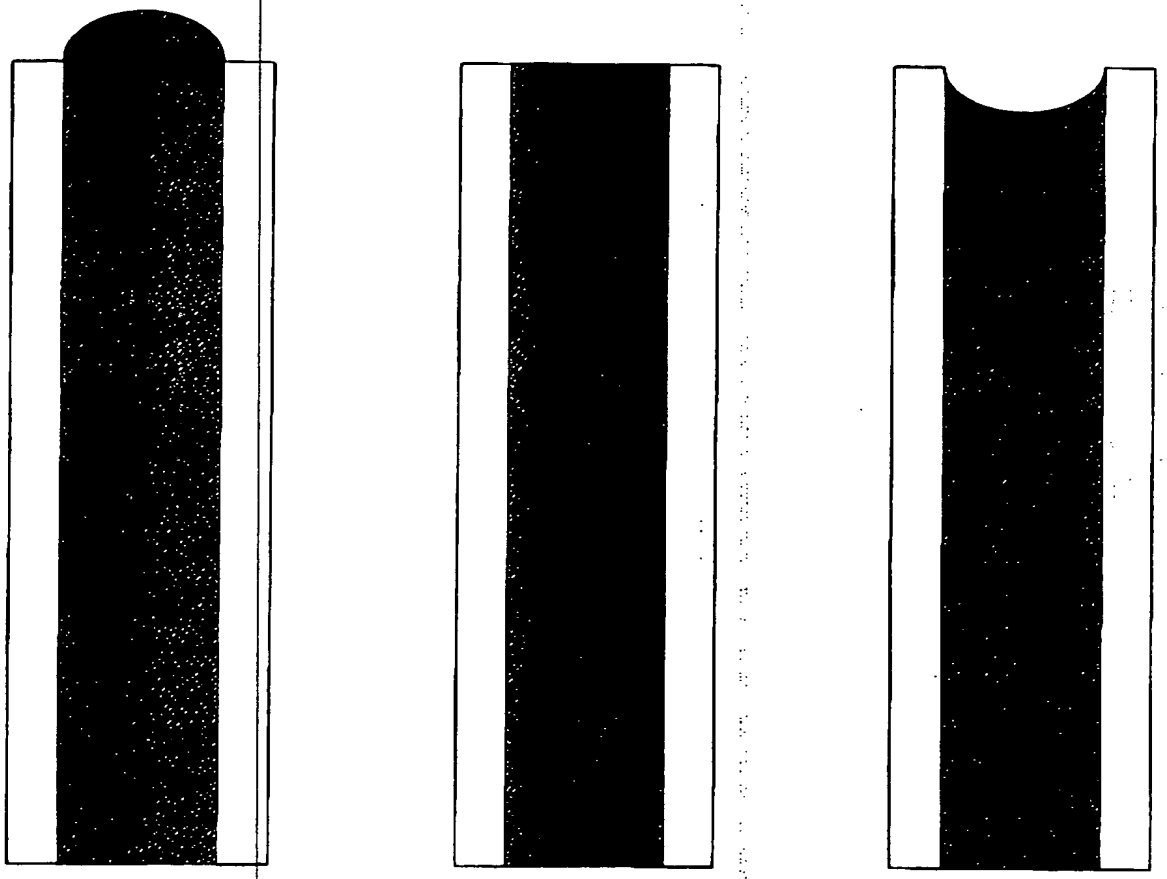


Fig. 1

Fig. 2

Fig. 3



a) b) c)

Fig. 4

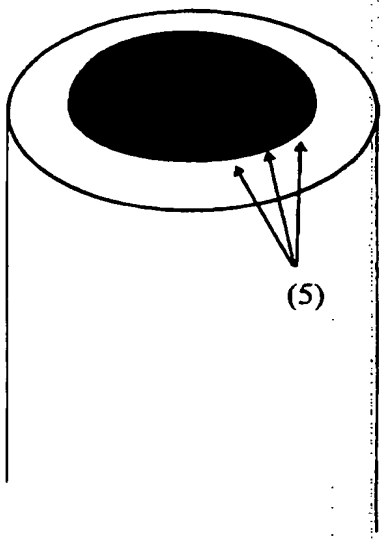


Fig. 5

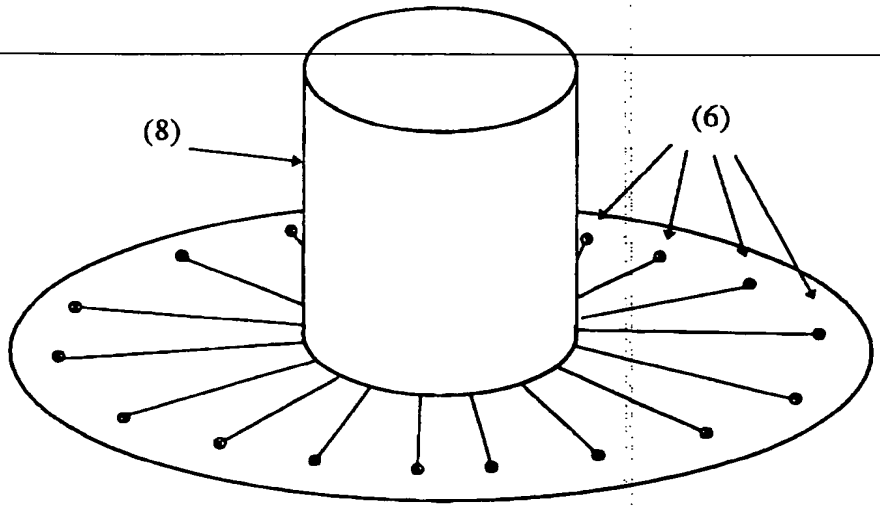


Fig. 6

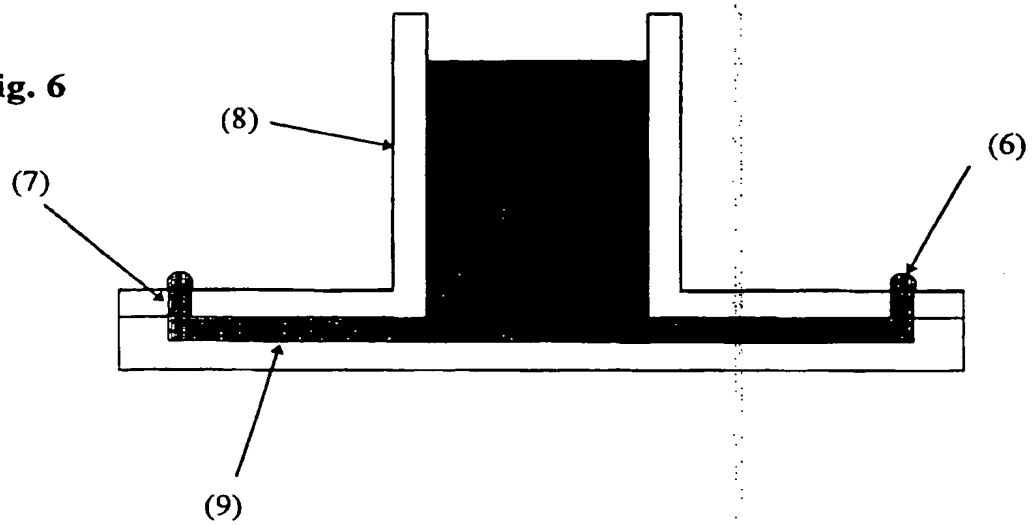


Fig. 7

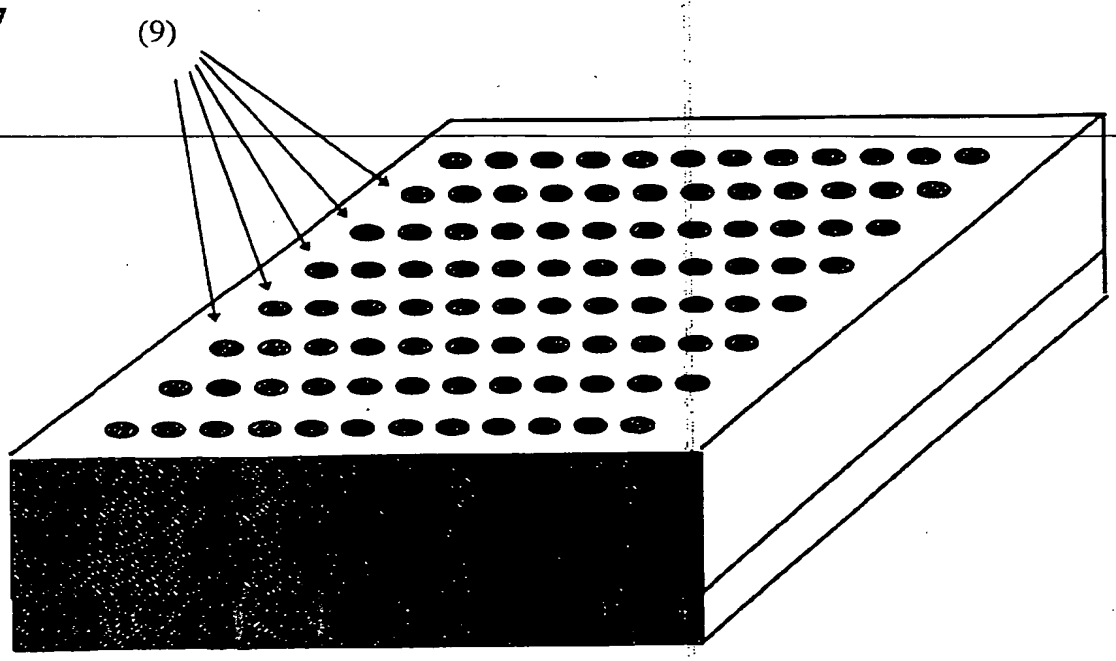


Fig. 8